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Landscape composition and climatic parameters significant in the spread of an invasive species (Pine wood Nematode)

Dissertação para obtenção do Grau de Mestre em Engenharia do Ambiente

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Resumo

Diversos estudos mostram que o número de invasões biológicas tem aumentado, e que a dispersão destas espécies da sua área geográfica para outros locais está directamente relacionada com o aumento do transporte e movimento de pessoas e mercadorias. O nematode da madeira do Pinheiro (*Bursaphelenchus xylophilus)* (NMP), a causa da doença da murchidão dos pinheiros, é um bom exemplo de uma invasão biológica. Esta espécie é nativa da América do Norte, onde não causa efeitos negativos nos pinheiros, mas tem-se tornado um problema na Eurásia.

O objectivo desta dissertação é compreender se parâmetros ambientais estão a determinar a mortalidade dos pinheiros bravos numa área afectada pelo NMP. O local de estudo está situado na zona centro de Portugal, onde a presença do NMP foi confirmada em 2009. Esta área é complexa em termos de topografia e solos, o que influencia a complexidade do clima e das condições ecológicas, sendo por isso um local ideal para estudar a interacção dos parâmetros ambientais e uma invasão biológica. Para avaliar a zona, diversos parâmetros ambientais foram analisados com recurso a SIGs e adicionalmente determinada a percentagem de árvores recentemente mortas em locais de amostragem determinados aleatoriamente.

A regressão binária logística indicou que a temperatura nos trimestres mais quentes é o parâmetro que melhor determina a probabilidade de mortalidade. Tendo em conta que os atuais modelos de alterações climáticas prevêem um aumento futuro da temperatura, os resultados obtidos no presente trabalho apontam para que os danos causados pelo NMP piorem nos próximos anos por causa da maior susceptibilidade dos pinheiros nestas condições.

Palavras-chave: *Bursaphelenchus xylophilus*, Invasões Biologicas, Sistema de Informação Geográfica, Parâmetros ambientais, Praga florestal.

Abstract

Studies show that the number of biological invasions has increased. The spread of species from their native range to other places is directly related to the increase in transport and movement of people and their goods. The Pine Wood Nematode (*Bursaphelenchus xylophilus*) (PWN), the causal agent of the pine wilt disease, is an example of this problem, originated from North America, has become a serious pest on Eurasia.

This dissertation aims to understand if environmental factors are determining tree mortality in an area where the PWN has been confirmed since 2009. The study area is located in the centreal part of Portugal, a complex area regarding the topography and soil making it also complex concerning climatic and ecological conditions, providing a good study case on the possible interaction between a biological invasion and local environmental conditions. In order to evaluate the study area several of environmental parameters were determined, and analyzed in GIS. Additionally it was determined the proportion of recently dead trees in randomly selected pine forests within the study area.

The Binary logistic model showed that temperature in the warm trimester was the only parameter that better predicted the mortality. Climate change models predict an increase in the temperature in the future, thus indicating that the susceptability of *Pinus pinaster to* the PWN will increase.

Keywords: *Bursaphelenchus xylophilus*, Biological invasion, Geographic Information System, environmental parameters, Forest pest.

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Abbreviation List

AWC – Available Water Capacity

FAO – Food and Agriculture Organization

GIS – Geographic Information System

GPS – Global Positioning System

IS – Intervention Sites

NFA – National Forestry Authority

NFI5 – National Forestry Inventory

PWN – Pine Wood Nematode

RDP – Rural Development Program

SAC – Spatial Autocorrelation

UN – United Nations

DGADR - Direção-Geral de Agricultura e Desenvolvimento Rural

1. Introduction

Studies show that the number of species moved from their native range has increased, and this is mostly because of the increased transport and movement of people and their goods, taking species beyond their geographic boundary (Lockwood *et al.,* 2007). With this comes an increase in the number of pest and invasive species that affect ecosystems worldwide (Campbell S., 2005). An example of this problem is the wilt disease caused by the Pine Wood Nematode (PWN) that has spread through Eurasia and has been confirmed in several countries that are geographically far away from each other, most likely due to the trade of forestry products (Futai, 2008).

To successfully protect plants and forests from biotic agents a coordinated international action is needed, due to the global dimension of this problem (FAO, 2011). The International Plant Protection Convention (IPPC) is an international agreement between countries to control pests and prevent their spread by implementing phytosanitary measures in order to ensure the quality of production and maintenance of ecosystem biodiversity, while permitting trade (FAO, 2011). Each country develops its national plant protection organization with phytosanitary measures that can be applied to forest products, prior, during and after import/export, measures that include inspections, different treatments and even quarantine (FAO, 2011). In Portugal the “Plano de Acção Nacional para o Controlo do Nemátodo da Madeira do Pinheiro” was implemented with several measures that would help manage and control this pest to ensure that it would not spread to other neighboring countries.

Even with all these measures the PWN has spread to other locations in Portugal, and to several other countries, indicating that more understanding and new ways to control this pest are necessary. In order to evaluate this problem, and more efficiently control it, it is crucial a good understanding of the factors involved in the invasive process and setting of the pine wilt disease, such as the biology of the pine host and the PWN, the environmental parameters that shape their ecosystem, and how these factors influence the mortality rate.

1.1. Objective

The main objective of this dissertation is to assess if environmental factors are determining tree mortality in an area where the PWN has recently spread out. For this, the environmental parameters involved, climatic and topographic, their interrelationship at the landscape level and the relation with the proportion of recently dead trees was assessed.

1.2. Organization

This dissertation is divided in seven parts. The first part addresses the increasing problem of biological invasions, the goals and the overall organization for this study.

The second part is a literature review focused on biological invasions, the way pests affect forests, how the pine wood nematode (PWN) acts, its geographical distribution and the effect it has had in Portugal.

The third part outlines the methodology that was adopted for the work and how data was collected and handled.

The fourth part presents the main results: some of the GIS maps generated from the different analysis as well as the statistical evaluation and models.

Then fifth part concentrates on the discussion of the results. In the sixth part conclusions are drawn on the viability of using GIS to detect and control pine wood nematode. The ideas of future work and the challenges faced during this study are also listed.

The last part presents all the quoted references made along the text.

2. Literature review

2.1. Biological Invasions

According to Williamson (1996) a biological invasion is the process by which an organism arrives somewhere beyond its previous range. But just the arrival of these species doesn´t guaranty that the invasion will be successful, and on average only 10% of them are able to get established in their new environment.

The invasion process is complex and depends on several factors. In order to be successful, the non-native species must pass at least three stages before they are able to inflict ecological or economic harm (Figure 1; Lockwood *et al.,* 2007) and while passing these stages they will have to overcome several ecological barriers (Williamson, 1996).

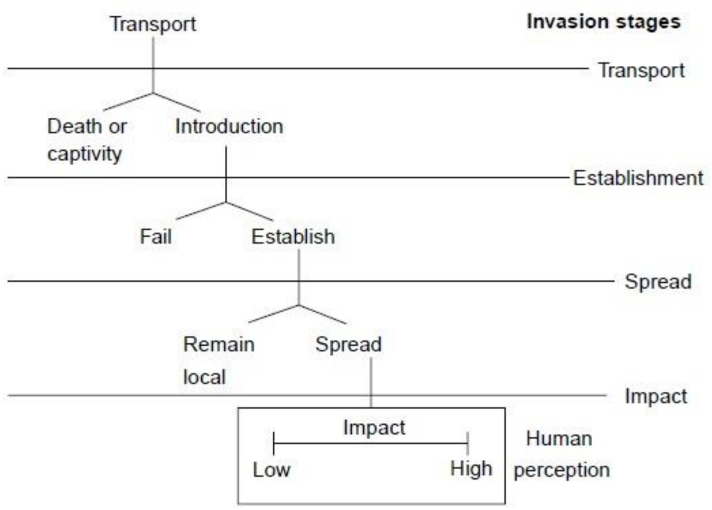

Figure 1 - Invasion process model. [Source: Lockwood *et al.,* 2007]

The invasion process starts with an individual that is transported from its native range to another location and then released. After this the non-native species must establish a self-sustaining population or they will become extinct. If they establish a viable population they must grow in abundance and expand their geographic range, otherwise they will have a local distribution with a low and stable population level. When the non-native species becomes widespread and abundant and causes ecological or economic impacts it is considered invasive (Lockwood *et al.,* 2007).

This biological invasion depends on a transport vector, the manner in which a species is carried along, and a pathway, the rout between the native region and the location of release (Lockwood *et al.,* 2007). Humans play a big role in the transport of these non-native species and are thus considered a transport vector, either introducing them intentionally (domestic animals and ornamental plants) or unintentionally (byproduct of the movement of other goods) (FAO, 2011).

The type of invasive species also has an influence in the effects it produces on the ecosystem. They have been defined as drivers or passengers but Bauer (2012) suggests a new definition: *back-seat drivers*. While drivers are the direct cause of the decline of native species and alter the ecosystem (MacDougall & Turkington, 2005) and passengers are a symptom of a problem in the ecosystem, back-seat drivers act synergistically with ecosystem change (Bauer, 2012).

2.2. Forest Ecosystems

Forests cover one third of the Earth's land mass but are disappearing at an alarming rate (UNEP, 2011), from 2001 through 2010 about 40 million hectares of primary forests were lost (FAO, 2010). Like in other countries, the Portuguese landscape has been altered along the years, due to the expansion of cities and human activities. In 2012 Portugal's forests were about 3 564 hectares, 30% of which were pine tree forests (INE, 2012).

Pinus pinaster is the pine species most affected by the PWN in Portugal (Goncalves 2014). It is distributed throughout the Portugal, from the North (Minho and Trans-os-Montes) to the Setúbal Peninsula (Forey 1996).It occurs in areas with mean annual temperatures that range between 14-15 ° C, with an absolute minimum temperature of 15 ° C and maximum of 40 ° C, and with a mean annual precipitation of 800 mm, with at least 100 mm in the summer (Alves, 1988). It is resistant to drought (Gonzalez, 1995), but very sensitive to spring frost (Oliveira, 2000). In Portugal it is rarely found in an altitude above 800 mm, due to snow fall (Loureiro, 1993), but it can be found in 900-1000 mm of altitude in oceanic climates and 700 mm in continental climate (Alves, 1988).

Forests are complex ecosystems that provide a series of services, denominated by the United Nations 2004 Millennium Ecosystem Assessment (MEA) as ecosystem services (UNEP, 2011), like carbon sequestration, that have economic benefits (FAO, 2011). But throughout the years there has been an increase not only in their destruction but also in the emergence of numerous pests and invasive species that have negative effects when introduced (FAO, 2011; Lockwood *et al.*, 2007), causing economic and ecological problems (Mooney *et al.*, 2005), such as the loss of native species and ecosystem services (Lockwood *et al.*, 2007).

The trade of forest products (wood products) is an important part of the global Economy, and also of the Portuguese economy, but the increase of global trade and the need to have faster and more efficient ways to transport wood products increases the risk of pest spread, contributing to the degradation of forest ecosystems around the globe. It is estimated that outbreaks of forest insects can damage 35 million hectares of forests annually (FAO, 2010a).

The forest sector is very important to the Portuguese economy (Webster & Mota, 2008) and the PWN has a direct effect on the value of the goods provided by forests. There is no data that relates the amount of money “lost” due to infected pine trees but it has been evaluated that from2001 to 2009 Portugal spent a total of 24 million euros to control the PWN. Other countries like Japan, where the PWN was first introduced as an invasive species 100 years ago, spend 10 million a year (FAO 2010, FAO 2011) to control the pest, without any hope of eradication, which illustrates the potential economic impact of this species.

2.3. The Pine wood Nematode

The Pine wood Nematode (PWN) *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) is the causal agent of the pine wilting disease (Mamiya, 1983). It is considered a threat to the pine wood forests for not only causing significant mortality, due to its high growth and spreading rate, but also because it devreases the commercial value of the trees (Webster & Mota, 2008). This disease was first reported in 1905 in south Japan, and in time it spread to most of the Japanese territory (Mamiya 1988, Futai, 2008). However, the causal agent, the PWN, was only discovered later in 1971 (Zhao *et al.*, 2008). In 1979 the PWN was detected in the United States of America and then in 1983 in Canada. Further studies showed that this nematode is native to North America thus not affecting the native pine species. Because of this, it was possible to conclude that the PWN was introduced from North America to Asia (FAO, 2010a). In 1982/83 the PWN was detected in China and in Taiwan (Zhao, 2008) and later in 1988 it was discovered in South Korea (Shin, 2008). In Europe it was firstly reported in Portugal in 1999, in *P. pinaster* located on the Setúbal Peninsula (Mota *et al.*, 1999; Mota & Vieira, 2008).

Despite several attempts to prevent the dispersal of the disease, in 2008 it was considered spread to all the country, and the Portuguese territory was considered a “Restriction Zone” (Autoridade Florestal Nacional, 2013). In 2009 it was detected in the Madeira Island (Vicente *et al.*, 2012), and in 2011, in the Galiza - Spain, becoming a real threat to European Pine forests (Vicente *et al.*, 2012).

The PWN was first described in 1972 by Mamiya and Kyohara and in order to successfully infect a pine tree it uses a vector, and is almost exclusively associated with pine sawyer beetles of the genus *Monochamus.* The PWN can associate with different species of *Monochamus,* but in Portugal the only known vector is *Monochamus galloprovincialis* (Oliver) (Sousa *et al.,* 2001).

The PWN life cycle is divided in a propagative and a dispersal cycle (Wingfield *et al.*, 1981). The propagative cycle happens in the resin canals (Mamiya, Y. & Kiyohara, T., 1972) of the infected trees and takes 4 to 5 days, in optimal conditions (Ishibashi & Kondo, 1977). The dispersal cycle occurs when the PWN larvae move to the respiratory system of the vector (FAO, 2007) and it emerges from dead trees, in May or June (Mamiya and Enda 1972). The beetle then transmits the nematode either when it feeds on shoots of healthy pine (primary transmission), or when the female lays eggs in freshly cut timber or in a dying tree (oviposition) (FAO, 2007). In the case of transmission by feeding, infected tree starts to show the symptoms of the disease, like the decrease in oleoresin flow and arrest of transpiration, two and four weeks after the infection, respectively. After this the trees become chlorotic and die in approximately 3 months (Mamiya, 1976). The dead tree will now attract mature cerambycid beetles that will use it to deposit their eggs, restarting the cycle between the two (Wingfield *et al.*, 1981).

Several countries around the globe implemented ways to manage and control the PWN. The European Union, including Portugal, started to implement rules in order to prevent the propagation of this pest in the affected countries, as well as preventing spreading to other countries (Autoridade Florestal Nacional, 2012). In order to control this invasive species, the Ministério da Agricultura, Mar, Ambiente e Ordenamento do Território, and the National Forestry Authority (NFA), have come up with a series of measures to help protect and minimize the dispersal risk, while maintaining viable forestry activity (Autoridade Florestal Nacional, 2012). This includes awareness campaigns about phytosanitary measures dictating the cut, process, handle and transport of wood in the affected areas. Complementary to this there is a regular monitoring of coniferous trees with symptoms of decay and also tree sampling. These operations are determined by the National Forestry Inventory (NFI5), in order to understand the dispersal of the PWN (Autoridade Florestal Nacional, 2012). Additionally a buffer zone of 20 km was established along the border with Spain, to prevent the spread of the PWN to other EU member-states (Autoridade Florestal Nacional, 2012).

The results from the monitoring operations from 1999 to 2011 identified several places as intervention sites (IS), with an increase on the number of these sites along the years. IS are locations where the presence of the PWN was confirmed or that have a potential risk of being infected by the PWN, and are therefore considered as such as a precaution. With these operations, 337 parishes were considered IS, of which only 256 had the presence of the PWN. The problem with this method is that once a parish is considered an IS, it will remain as such even if later it is found to be disease free, contributing to the increase in the numbers of sites along the years. (Autoridade Florestal Nacional, 2012).

When an infected tree is detected it has to be removed and transported to an authorized destination. In 2011, 900 thousand trees were identified and eliminated, 55% of those located in the buffer zone, considered by the European Committee as a priority site (Autoridade Florestal Nacional, 2012).

3. Methods

3.1 Study area

The study area chosen for this dissertation is located in the central part of Portugal and has an area of 5 632 km^2 (Figure 2). This area was selected because the PWN was detected here, starting in 2008 (Vicent et *al.* 2008) and preliminary surveys pointed to significant mortality of the P.*pinaster* forests. This is a complex area as regards to topography and soil type. The large variability in altitude promotes variations, within short distances, in slope, aspect, rain, shading and exposure to wind. Thus, it is a complex area as regards to micro-climatic and ecological conditions.

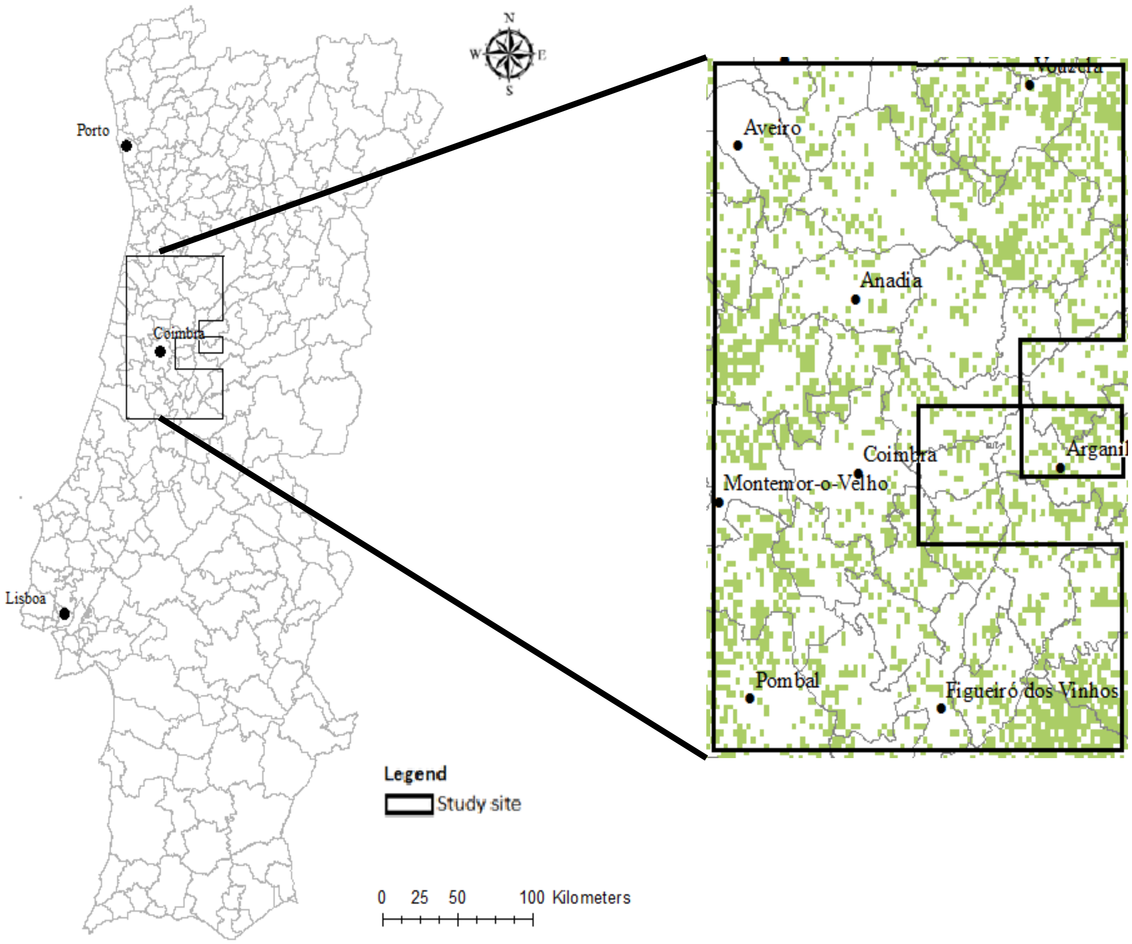

Figure 2 - Study area location (left) and *Pinus pinaster* distribution in the study area (right) [Fonte: Inventário Florestal Nacional (IFN)].

Altitude of the study area varies between 0 m and 1203 m (Figure 3), lower values found on the western part of the study stite and higher values in the east.

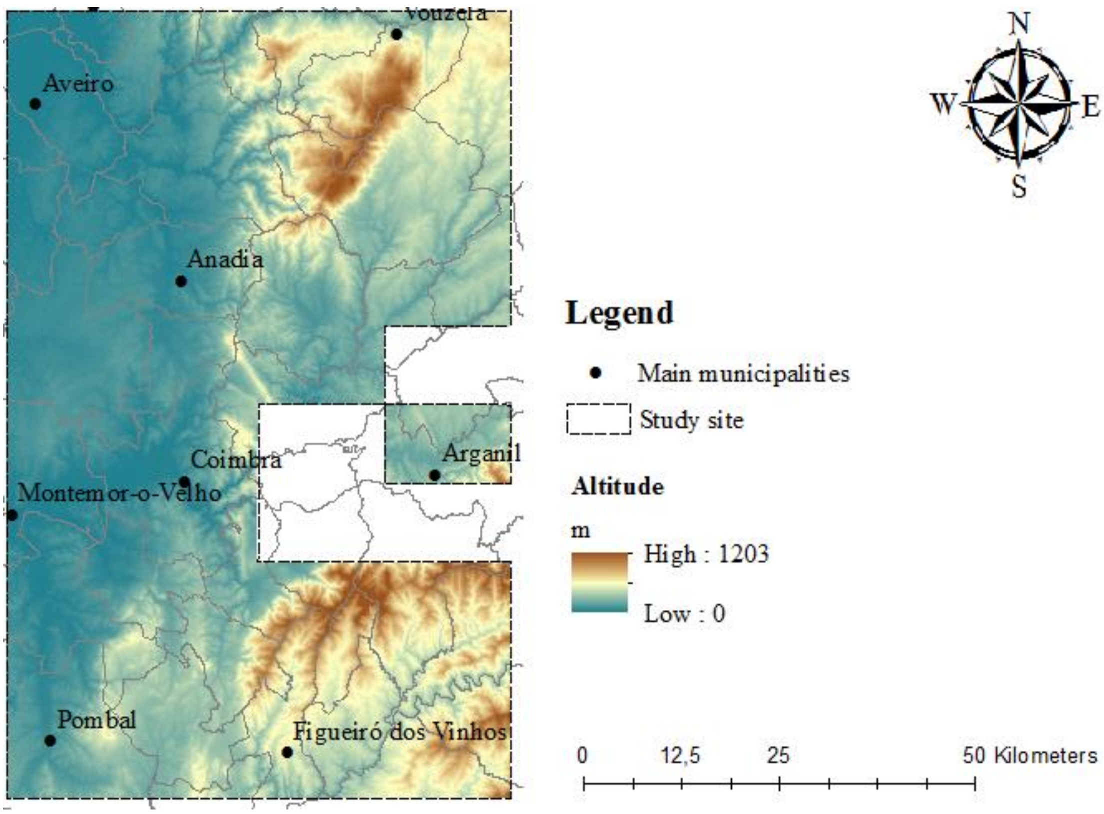

Figure 3 – Altutude (m) in the study area.

Slope in the study area (Figure 4) varies between flat areas and around 32% in mountainous areas (Figure 4).

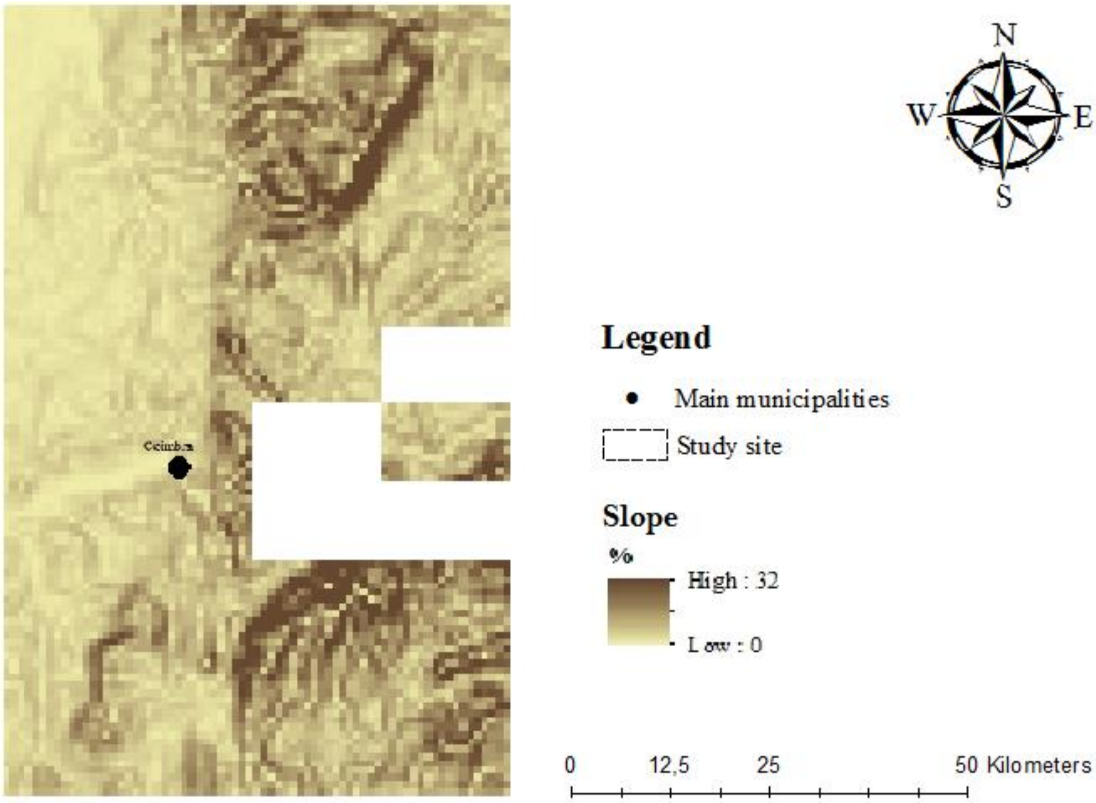

Figure 4 – Slope (%) in the study area.

Annual precipitation (Figure 5) varies from 855 mm in the south west to 1522 mm in the eastern mountainous areas, especially in the northeast.

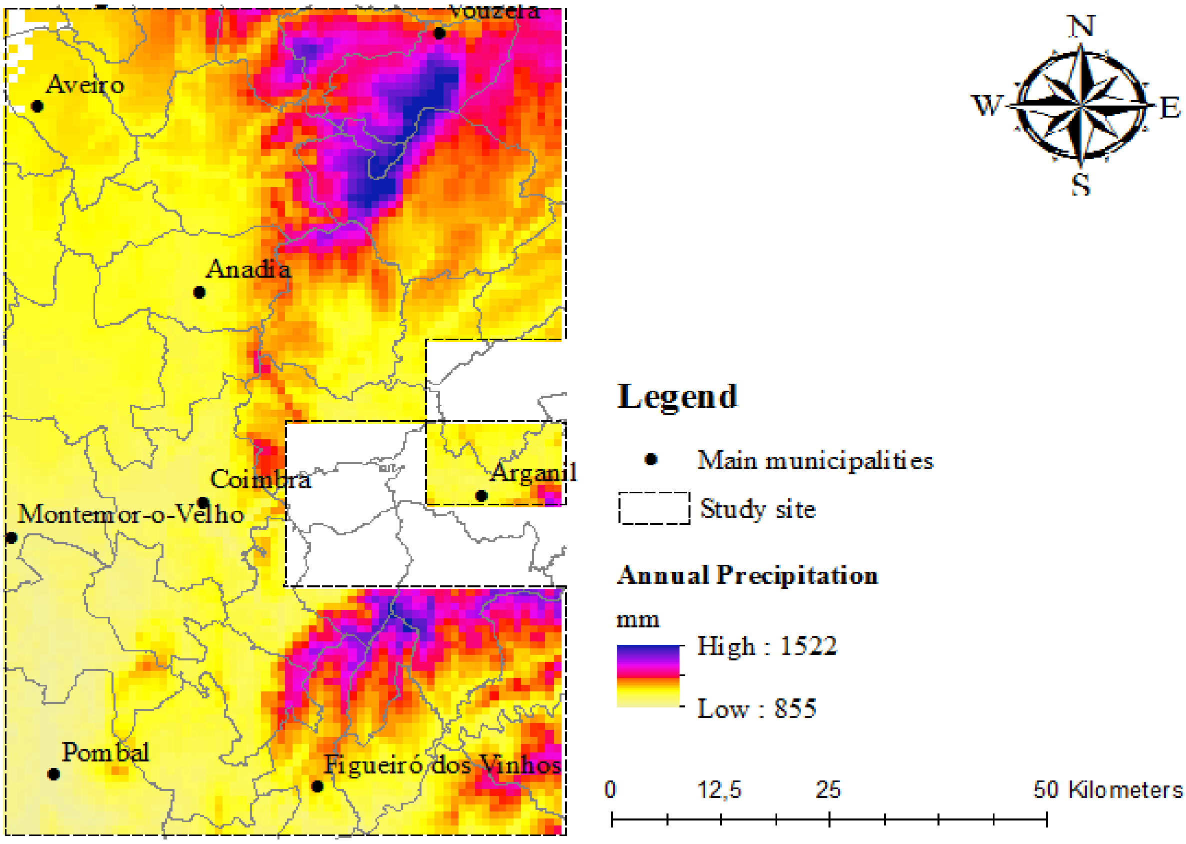

Figure 5 - Mean Annual Precipitation (mm) in the study area.

Average annual temperature (Figure 6) varies from 8 ºC in the East to 14 ºC in the southwest, near Coimbra. It reaches a maximum (23.2 ° C) in August and minimum (4.6 ° C) in January.

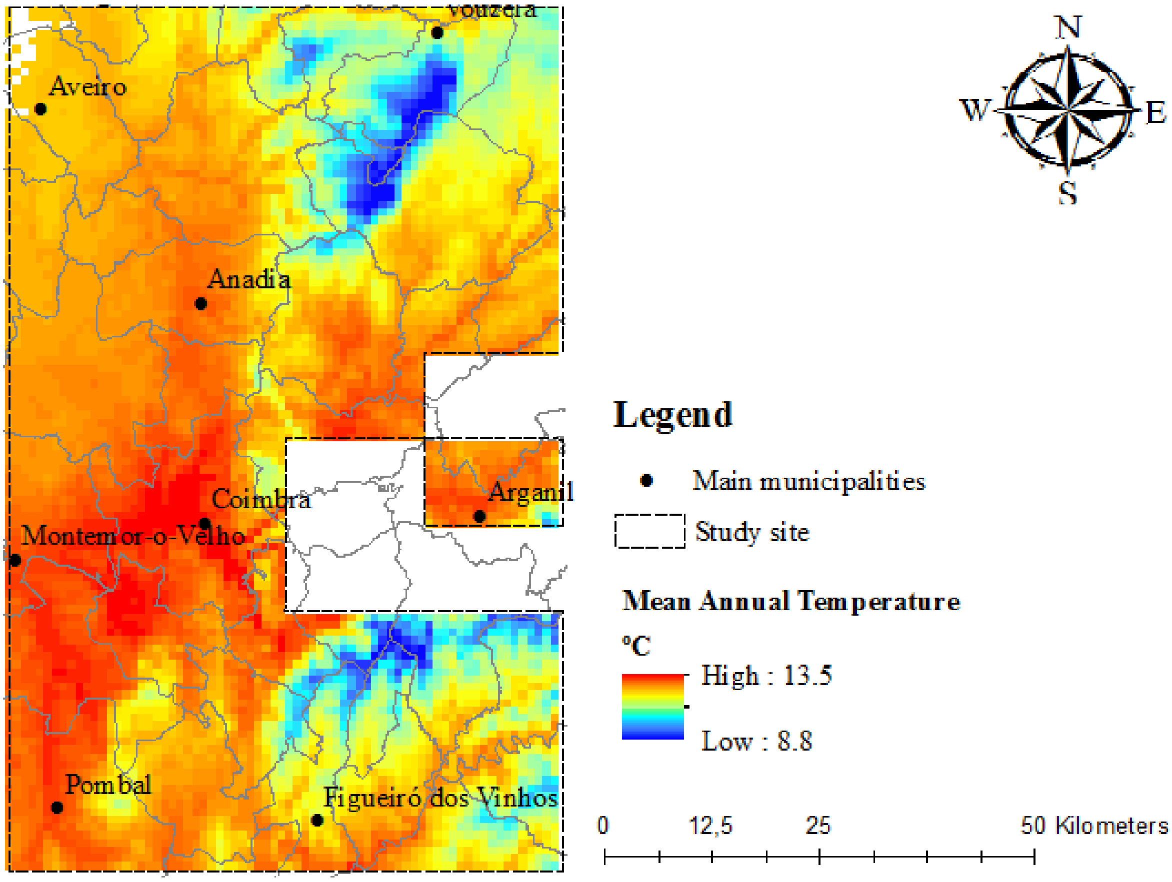

Figure 6 - Mean annual Temperature (ºC) in the study area.

Available Water Capacity (AWC) (Figure 7) ranges from 0 mm to 1034 mm and has a complex variation in the study area because it depends on the soil type.

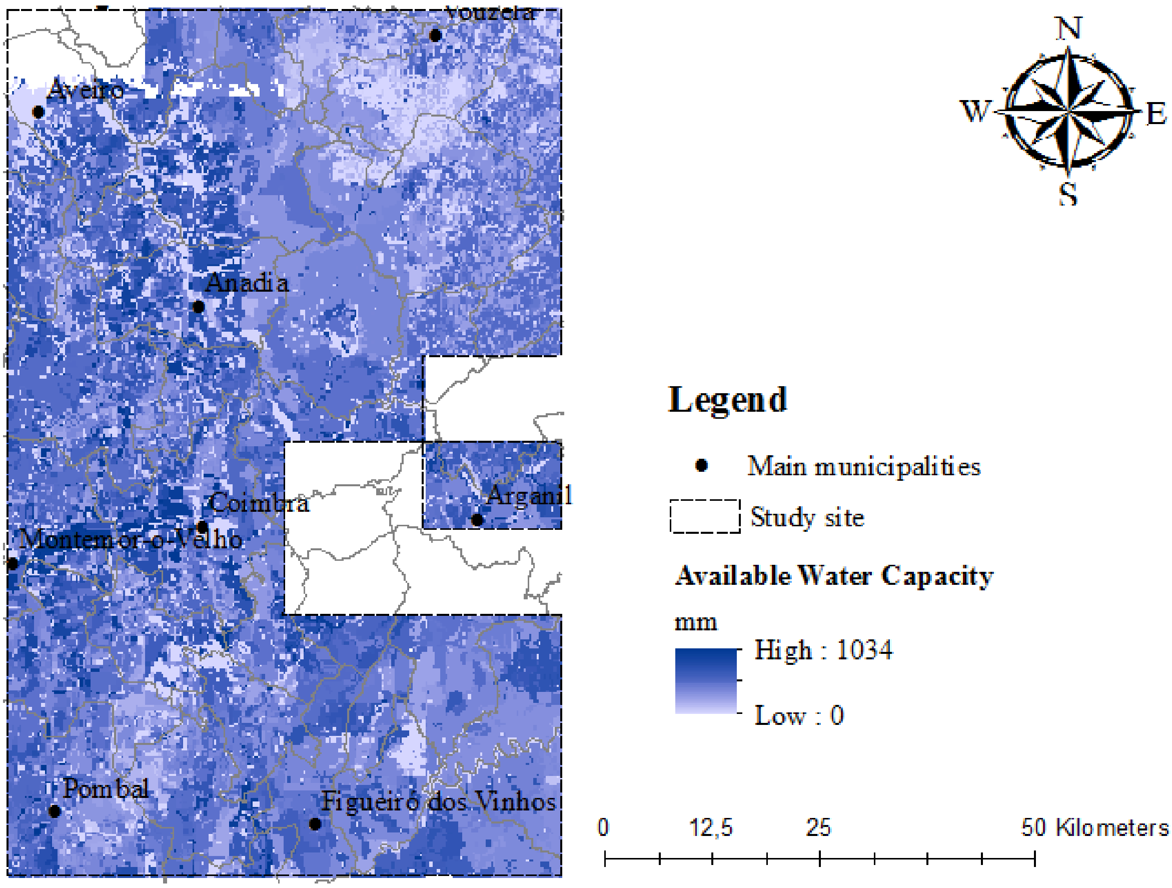

Figure 7 - Available Water Capacity (AWC) (mm) in the study area. The soil types found in the northwest area of the study area were not considered because there was no data for their AWC values.

Potential evapotranspiration (PET) (Figure 8) varies from 545.9 mm to 719.8 mm. The lowest values are found in the higher altitudes and highest values in the southwest part of the study area.

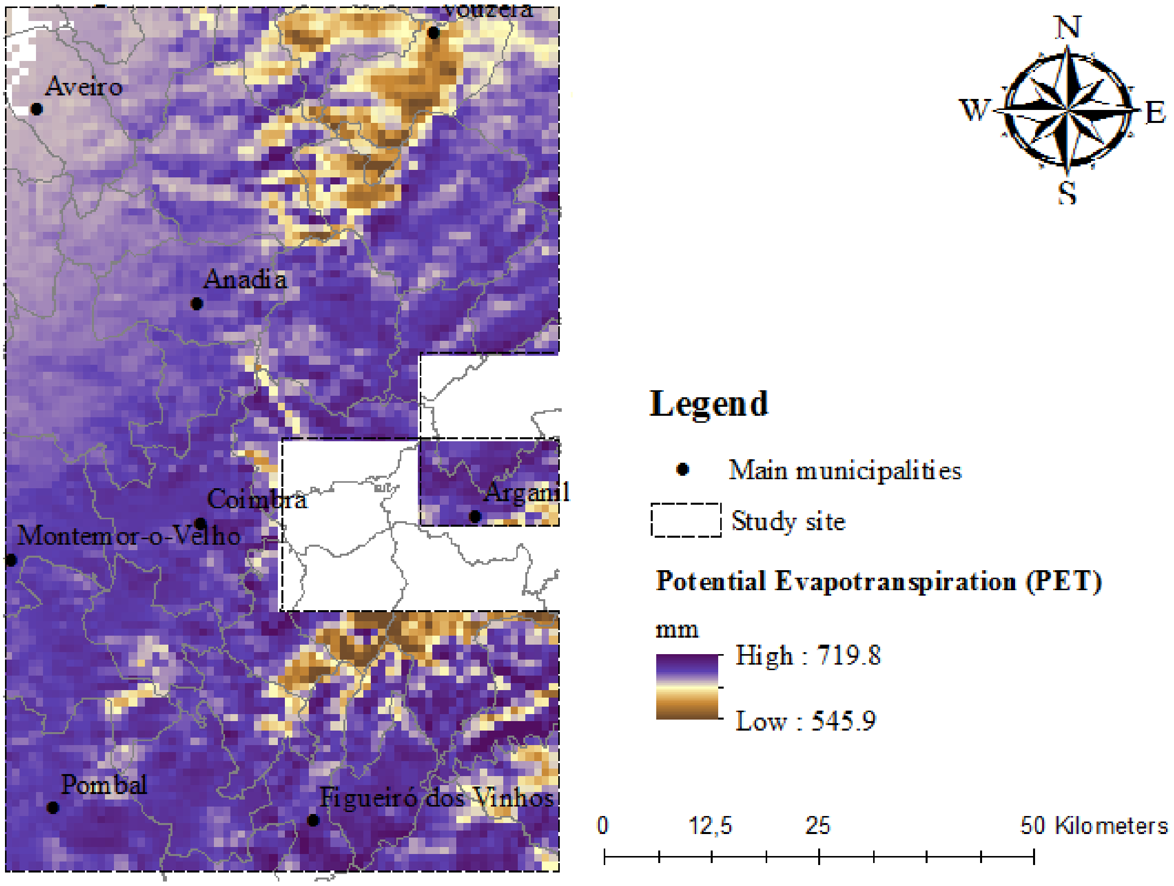

Figure 8 - Potential evapotranspiration (PET) (mm) of the study area.

Actual Evapotranspiration (AET) (Figure 9) ranges from 410 mm in eastern mountainous areas to 650 mm in western flat areas.

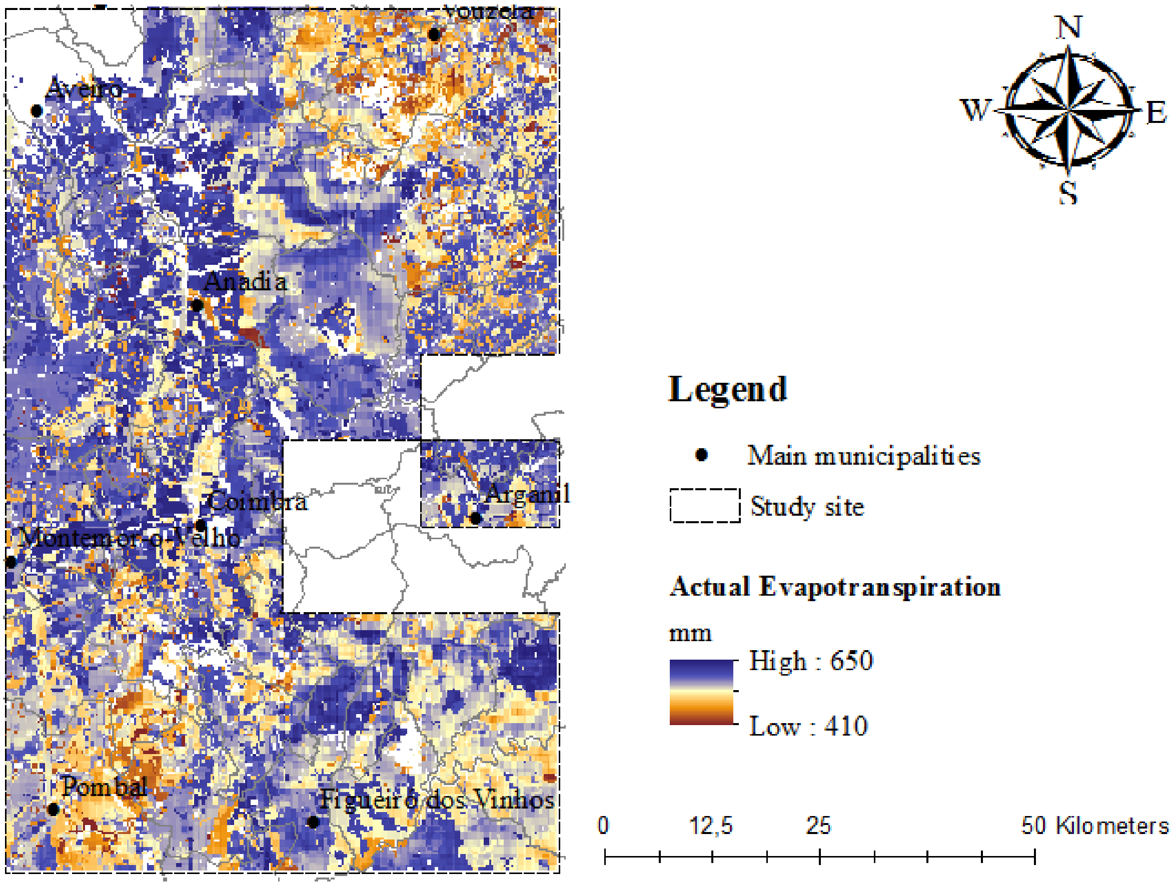

Figure 9 – Actual evapotranspiration (AET) (mm) in the study area. Upper left corner of the study area has no values because of the AWC values that were used to generate this map.

The climatic conditions in the study area are very important factors because they can affect the conditions of the pine trees and thus their susceptibility to disease and pests such as the PWN. In the study are different types of climate are felt (Figure 10).

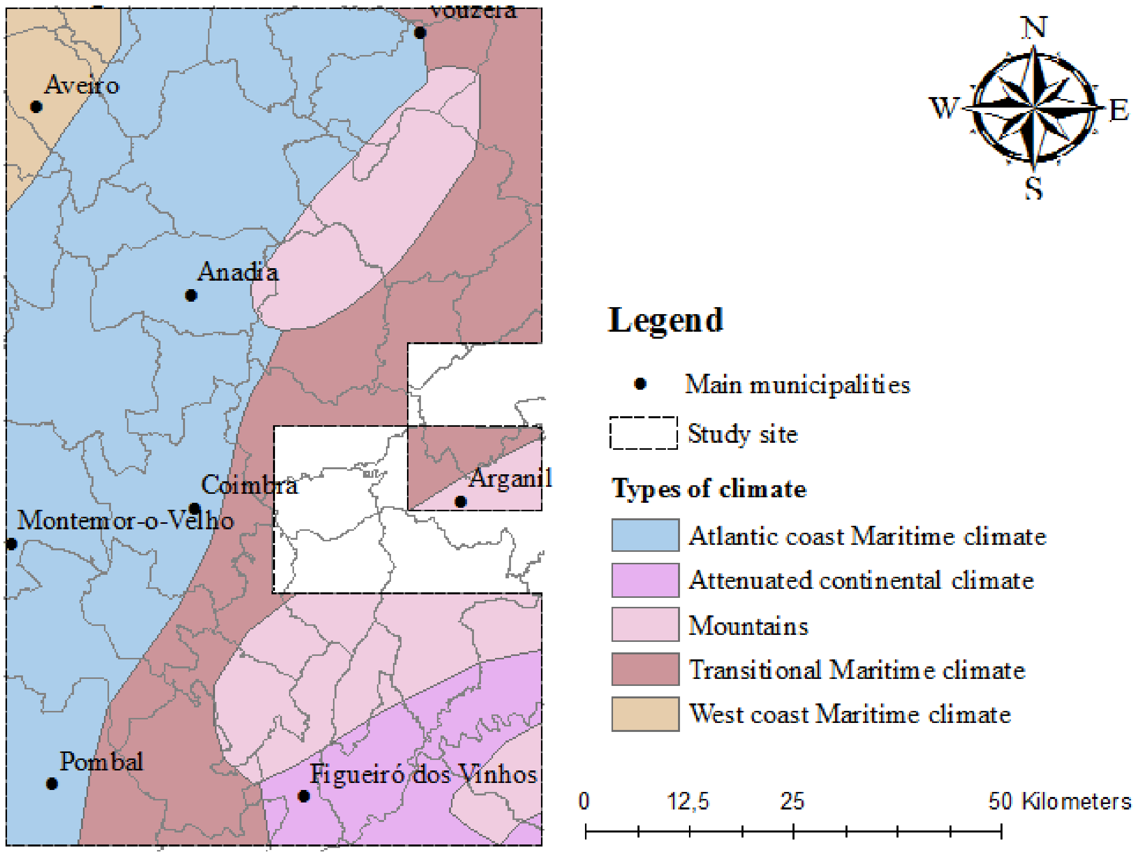

Figure 10 – Climatic regions present in the study area.

The study area lies in a transitional area between maritime climate and continental climate. The western part of the study area is influenced by the West coast Maritime climate and Atlantic coast Maritime climate while the eastern part is influenced by the Attenuated continental climate and in the central part the Transitional Maritime Climate is felt. Mountains mark their presence as regards to climate conditions, increasing precipitation and lowering temperature. (Daveau, 1985)

3.2 Sampling design and data collection

The development of the PWN and the incidence and severity of the wilting disease depend on the environmental conditions. To evaluate the parameters in the study area and the relationship between them a series of maps and *in situ* observations of the mortality were needed.

Environmental parameters

Most of the environmental parameters used in the present thesis (Table 1) were obtained from the WorldClim data base (www.worldclim.org), a set of global climate layers (in raster format) with a spatial resolution of about 1 square kilometer. Information in this database refers to the period between 1950-2000.

Table 1 - Environmental parameters, resolution/scale, format, georeferencing system and source.

Variable	Spatial Resolution/scale	Format	Georeferencing System	Source
Annual Precipitation (Prec)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Worldclim
Precipitation in the humid trimester (Prec_humid)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Worldclim
Precipitation in the dry trimester (Prec_dry)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Worldclim
Precipitation in the warm trimester (Prec_warm)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Worldclim
Precipitation in the cold trimester (Prec_cold)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Worldclim
Annual Temperature (Temp)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Worldclim
Temperature in the humid trimester (Temp_humid)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Worldclim
Temperature in the dry trimester (Temp_dry)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Worldclim
Temperature in the warm trimester (Temp_warm)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Worldclim
Temperature in the cold trimester (Temp_cold)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Worldclim
Soil type	1/25 000	Vectorial	Lisboa Hayford Gauss Igeoe	SROA/ CNROA
Altitude (Alt)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Calculated
Radiation (Rad)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Calculated
Slope	1-Km	Raster	Datum WGS-1984, latitude/longitude	Calculated
Available Water Capacity (AWC)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Calculated
Potential Evapotranspiration (PET)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Calculated
Moisture “supply-demand” (PE)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Calculated
Actual Evapotranspiration (AET)	1-Km	Raster	Datum WGS-1984, latitude/longitude	Calculated

Slope and radiation maps were computed in ArcGis 10.1 based on the altitude raster. Soil maps were provided by Direção-Geral de Agricultura e Desenvolvimento Rural (DGADR). They were supplied in raster (image) format and were digitalized to vector (polygon) format depicting soil family type and percentage. Each polygon may include information of up to three different families of soil. It was possible to gather information for the whole study area regarding all the environmental parameters except for soil type. For about 12% of the area it was not possible to have soil maps due to the fact that they were never published. Thus, in the maps presented in this study some white rectangular areas can be seen that correspond to missing information.

In total 17 maps were produced for each environmental parameters, and all of these were transformed to the European Terrestrial Reference System 1989 (ETRS 89), an European Union recommended frame of reference for geodata for Europe.

Available water capacity (AWC) expresses the quantity of water present in the soil available to plants (Costa, 1995). It is an important edaphic factor because it influences site water balance, which in turn affects plant activity. Thus it is a critical parameter that needs to be determined in the context of ecological studies. However this parameter was not originally included in the soil maps and in order to generate AWC data several procedures were followed. First all the soil families represented in the study area were identified, 223 families were accounted for.

Then soil texture was evaluated because it determines the relative capacity of soil to hold water that is available for uptake by plants. Texture can be assessed through data from the different soil particle-size classes (sand, silt, and clay) (Costa, 1995). Information regarding the fraction of the different soil particle-size classes of the various soil horizons was obtained for these families, mainly from Cardoso (1965). Since Cardoso (1965) did not have information regarding all the families, the textural class for several soil families was retrieved from the DGADR website (http://www.dgadr.mamaot.pt/nota-explicativa). Even after this literature review information for 58 families of soil was not found and subsequently the corresponding areas were considered as No Data.

Soil families for which it was possible to find fraction values for soil particle-size classes a well-established soil textural triangle diagram, adopted by the US Government, was used in order to determine soil texture. Concerning soil families whose textural class was given by the DGADR website, a different procedure was used because textural information in this case came from a different diagram, a soil textural triangle diagram used for Portuguese soils. Thus, it was necessary to apply a conversion scheme between the two different diagrams (Figure 11,Figure 12).

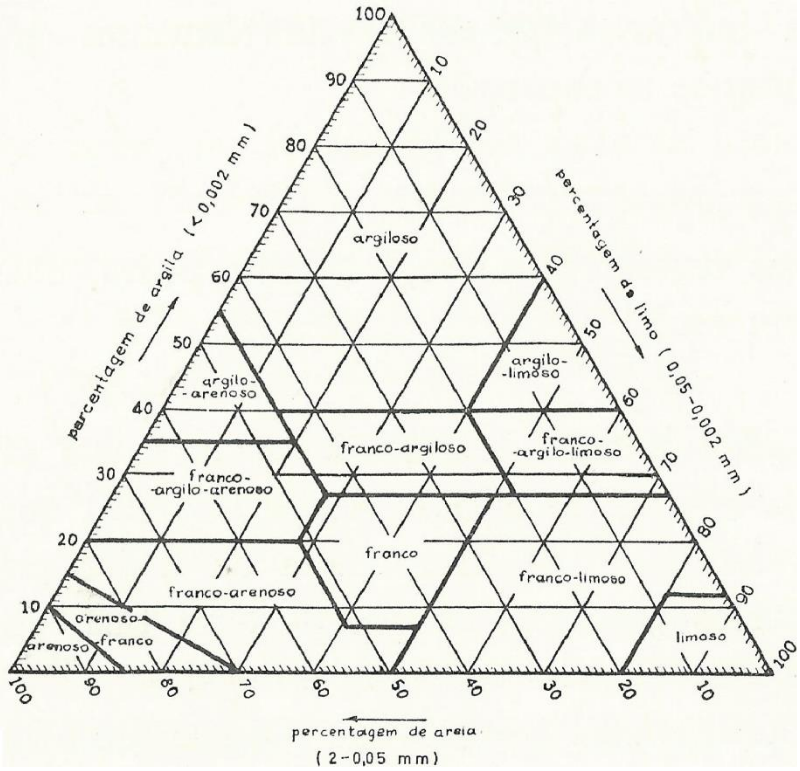

Figure 11 - Soil textural triangle adapted to international limits of the granulometric fractions, by M.Pereira Gomes e A. Antunes da Silva. [Source: Costa, B., 1995].

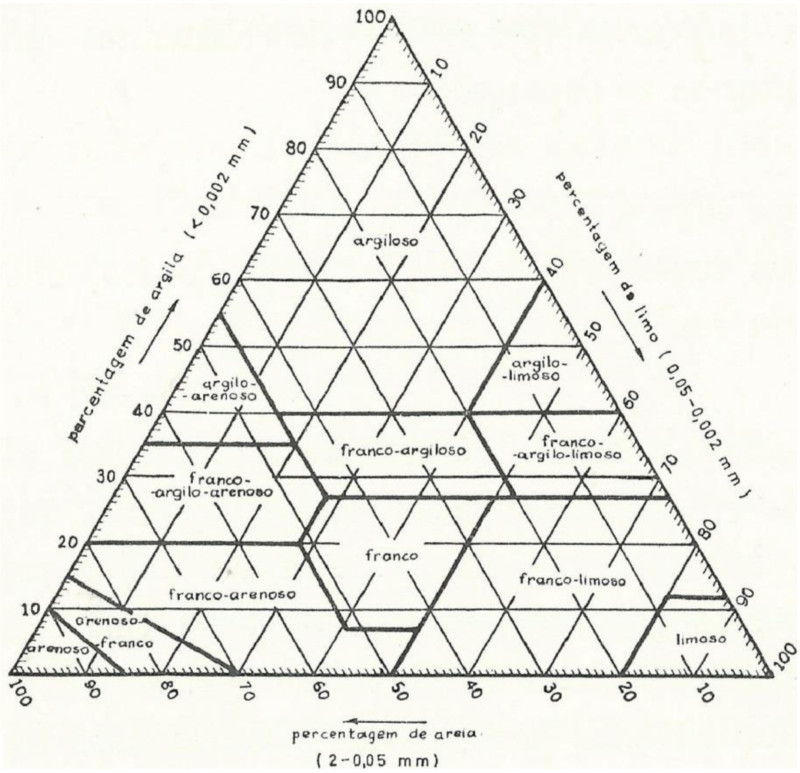

Figure 12 - Soil textural triangle adopted by the Department of Agriculture of the United States of America. [Source: Costa, B., 1995]

AWC was determined for each horizon of the different soil families using a table by Allen *et al.* (1998; 2007) which relates texture class with soil water content. To determine final AWC for a given soil family AWC values for the different horizons were summed. AWC calculation in cases where more than one family of soil was present was based on the representativeness of the different families. Final soil AWC value was determined for a depth of 0-150 cm because, in general, pine trees have deep roots. All soil maps were converted into raster format for further processing.

Dyer (2009) developed the Water Balance Tool that enables the calculation of evapotranspiration and moisture stress using climatic, topographic and edaphic data in a GIS. This model runs as a Toolbox in ArcGis with maps in raster format.

Firstly the model calculates potential evapotranspiration (PET), a measure of moisture demand, which is the amount of water that can be evaporated and transpired from a vegetated surface if water is not a limiting factor. The model uses the Turc method (Equation 1) where PET is the monthly potential evapotranspiration in mm, T represents the mean monthly temperature in ºC, and R_S indicates monthly global radiation received at the earth's surface, in cal/cm^{-2}.

$$\mathrm{PET} = 0.013 \times \left[\frac{\mathrm{T}}{(\mathrm{T}+15)}\right] \times (\mathrm{R_s} + 50) \qquad \text{Equation 1}$$

PET maps were produced for each month of the year. These maps enable the calculation of the moisture "supply-demand" (P-PE) maps that compare the PET values with the respective precipitation for each month. Positive values mean precipitation is enough to meet the plants moisture needs and the negative values indicate that the plants use the soil moisture to meet their needs (Dyer, 2013).

The next step consists in the calculation of the soil moisture storage (monthly values, representing storage on the last day of the month) by dividing monthly values by the number of days in the month (Dyer, 2013). In this part of the model the soil AWC maps are used as input.

With the storage maps monthly actual evapotranspiration (AET) is computed using an elevation model (DEM), average monthly precipitation maps, PET maps and P-PE maps. The Water Balance Tool produces twelve AET maps, one for each month of the year. Then in ArcGis the annual AET was computed summing all the monthly maps. This final map was used in the statistical analysis.

Field surveys

In order to evaluate maritime pine tree (*Pinus pinaster*) mortality in the study area a set of points for field survey evaluation were randomly generated with the help of ArcGis. Field surveys were conducted from July to December 2013. In each sampling point total basal area and basal area of dead standing trees were measured, at three spots along a 200 m transect, with the help of an English BAF 10x wedge prism. The wedge prism is used to scan along the basal area. It refracts the light at a specific offset angle creating an optical illusion of the tree trunk (Figure 13).

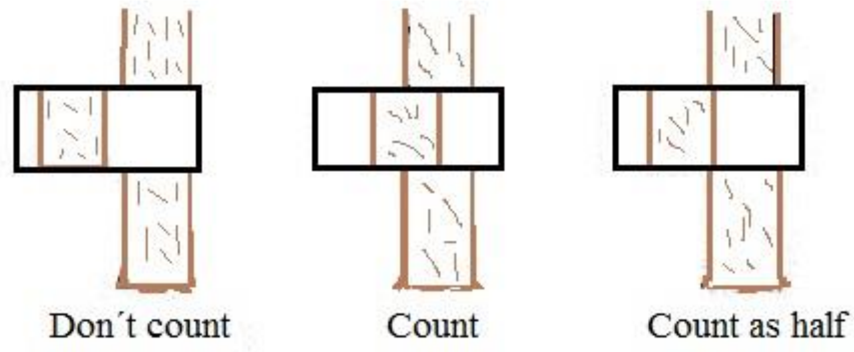

Figure 13 - Optical illusion created by the wedge prism when calculation basal area.

If the offset portion of the trunk viewed through the prism appears connected to the main trunk of the tree, then it is countable, if it appears to be borderline it counts as half a tree and if it looks completely away from the main trunk it is not countable. It is then necessary to multiply the total number of trees by the basal area factor of the prism, in this case 10. Tree mortality was calculated as the proportion of dead standing basal area over the total basal area.

Some of the original sampling points had to be later eliminated since they were not in pine tree areas and could not be used for analyses. In the end 51 sampling points (Figure 14) remained and were used in the statistical analysis. For these points the values for all environmental parameters were extracted from the maps.

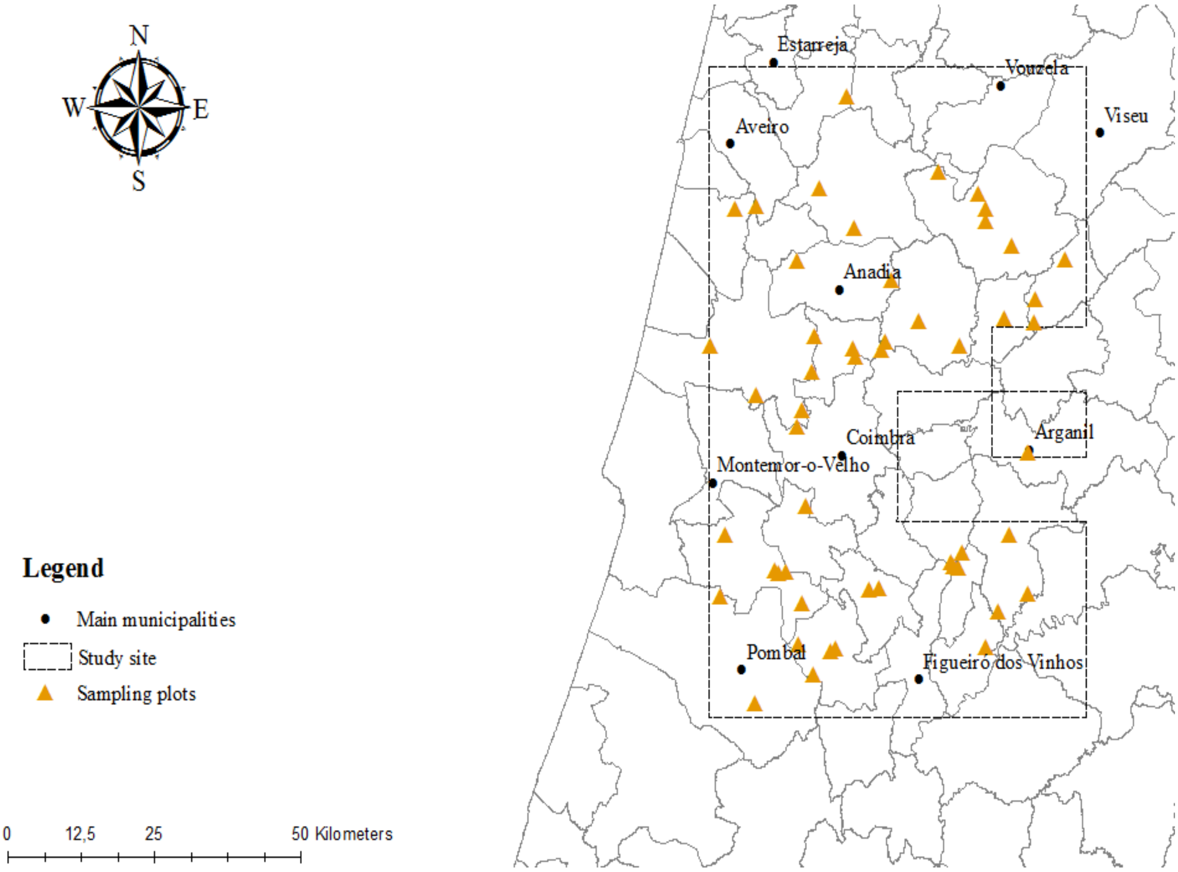

Figure 14 - Location of the sampling points.

3.3 Statistical analysis

Univariate Moran's I index, that indicates the statistical significance of spatial autocorrelation (SAC), was calculated for all parameters in the study area. It was considered to occur SAC when the p-values were lower than 0.05 and the z-scores were higher than 1.96 (Vu *et al.*, 2014). Positive Moran's I index values for small distances and negative values for longer distances also indicate the existence of SAC. This index was computed in IDRISI software for all the raster images corresponding to the different parameters.

Pearson correlation coefficient was calculated between mortality and the 17 environmental parameters to understand if these parameters were independent or correlated. If the results indicated that two parameters were correlated more than 90%, then the one that presents higher correlation with more parameters has to be removed, because they make it difficult to distinguish which parameter is important in predicting the outcome.

The parameter percentage of mortality was not normally distributed and could not be normalized, and therefore could not be used for statistical analysis. Thus a Binary logistic regression was used with a binary parameter presence/absence of mortality as the dependent variable.

Then logistic binary regression models were developed to evaluate the relationship between the response variable, mortality, expressed as 1 for presence and 0 for absence, and its predictors, the 17 environmental parameters (independent variables). The logistic model is more suitable to explain presence/absence data (Augustin *et al*, 1996), because it combines the independent variables to estimate the probability of an event, in this case mortality (Equation 2).

$$ln\left(\frac{p}{1-p}\right) = \beta_0 x_0 + \beta_1 x_1 + \cdots + \beta_k x_k \qquad \text{Equation 2}$$

Where p is the probability of the event and varies between 0 and 1.

In computing the logistic regression the Backward Likelihood method was used in SPSS because it eliminates step by step the parameter with the highest p-value until only the significant and better fit remains to determine mortality.

The Receiver Operating Characteristic (ROC) statistics was calculated assuming that the distribution is non-parametric, with a confidence interval of 95%, to validate the best fit model for the binary logistic (Swets, 1988).

4. Results

With the values of the percentage of tree mortality observed in the sampling plots a map was generated to help visualize the distribution of this data (Figure 15).

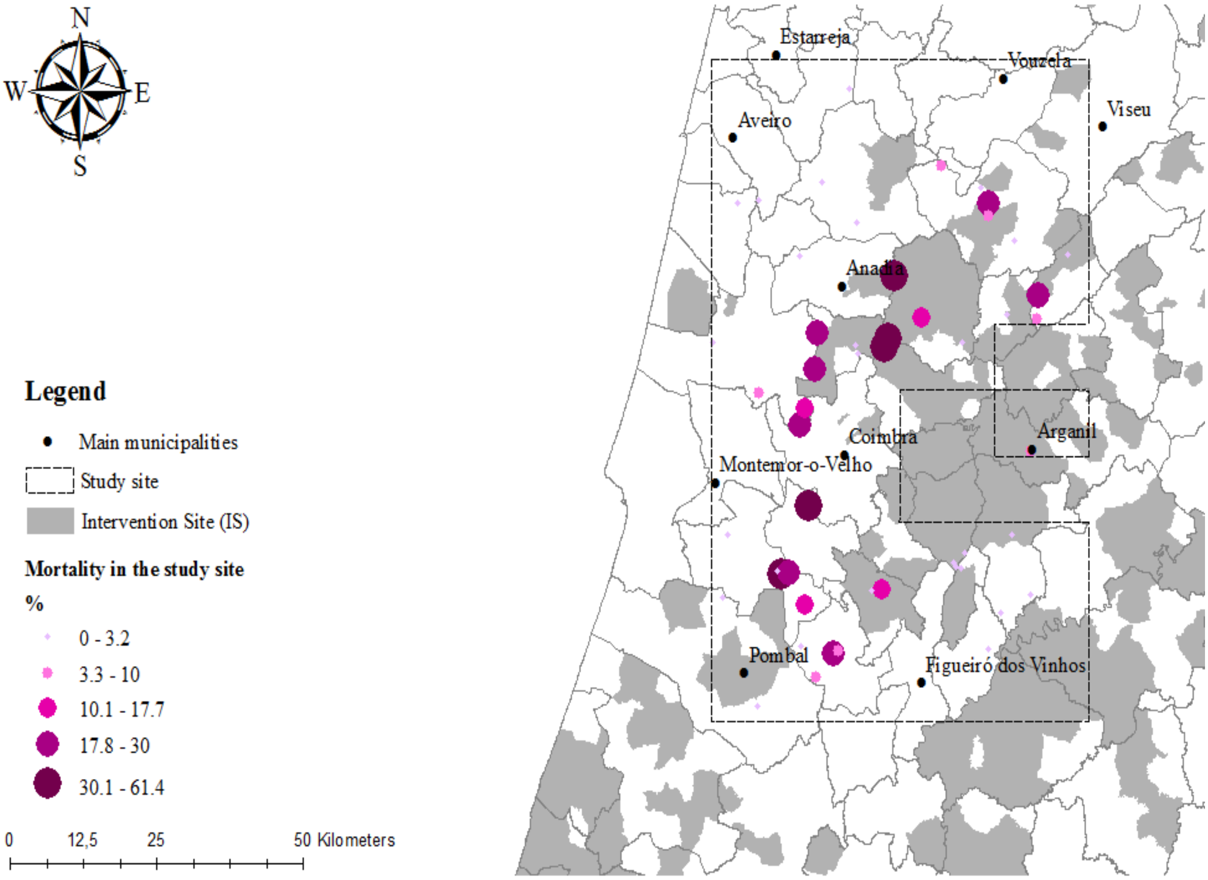

Figure 15 - Percentage of mortality distribution along the study area.

Spatial autocorrelation

Univariate Moran’s I index values indicated that all parameters exhibit z-scores higher than 2.58, indicating a clustered pattern and a significant positive spatial autocorrelation at 95% level.

Moran’s I coefficients were only viable for computation from a distance of about 15 km, the lowest distance found between two sampling plots in the study area.

Pearson Correlation

Pearson correlation coefficients between all parameters are presented in Table 2. At a significance level of 0.01 many parameters were found to be highly correlated. None of the parameters showed a high correlation value with mortality and only two showed statistical significant correlation at a significance level of 0.05: Temperature in the warm trimester and Temperature in the dry trimester. Radiation, Slope, Available Water Capacity (AWC), Potential Evapotranspiration (PET) and Actual Evapotranspiration (AET) are not significantly correlated a a significance of 0.05 with any other parameters. The highest Pearson correlation coefficient (0.999) was obtained between Precipitation in the humid trimester and Precipitation in the cold trimester. Other parameters also showed high positive correlation values, such as Temperature in the dry trimester and Temperature in the warm trimester (0.997), Precipitation in the cold trimester and Annual Precipitation (0.997) and Moisture “supply-demand” (PE) with both Annual Precipitation (R= 0.994) and Precipitation in the humid trimester (R=0.991).

Table 2 - Pearson correlations between all pairs of parameters. P-values <0.01 and 0.01<p<0.05 .

Variable	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1 Mortality	1.00																	
2 Altitude	-.136	1.00																
3 Radiation	-.188	.733	1.00															
4 Slope	-.152	.668	.501	1.00														
5 Annual Temperature	.213	**-.949**	-.740	-.679	1.00													
6 Temperature in the cold trimester	.108	**-.971**	-.759	-.680	**.961**	1.00												
7 Temperature in the warm trimester	.358	-.624	-.497	-.511	.816	.636	1.00											
8 Temperature in the humid trimester	.052	**-.967**	-.736	-.646	**.911**	**.975**	.524	1.00										
9 Temperature in the dry trimester	.358	-.595	-.480	-.481	.792	.603	**.997**	.491	1.00									
10 Annual Precipitation	-.150	**.907**	.725	.689	**-.972**	**-.962**	-.775	**-.899**	-.749	1.00								
11 Precipitation in the cold trimester	-.141	**.937**	.738	.695	**-.982**	**-.978**	-.759	**-.925**	-.732	**.997**	1.00							
12 Precipitation in the warm trimester	-.166	.601	.540	.552	-.771	-.725	-.765	-.611	-.752	.875	.832	1.00						
13 Precipitation in the humid trimester	-.164	**.926**	.735	.691	**-.983**	**-.971**	-.779	**-.912**	-.753	**.998**	**.999**	.848	1.00					
14 Precipitation in the dry trimester	-.084	.819	.672	.655	**-.902**	**-.902**	-.729	-.832	-.708	**.974**	**.957**	**.938**	**.960**	1.00				
15 AWC	-.182	-.061	-.157	-.015	.049	.070	-.028	.101	-.026	-.034	-.044	.001	-.038	-.059	1.00			
16 PET	.240	-.684	-.302	-.533	.775	.688	.760	.625	.743	-.749	-.746	-.644	-.753	-.684	-.133	1.00		
17 PE	-.169	**.895**	.683	.653	**-.965**	**-.946**	-.792	-.880	-.767	**.994**	**.988**	.882	**.991**	**.970**	-.020	-.782	1.00	
18 AET	-.068	-.175	-.094	-.094	.140	.118	.074	.166	.078	-.060	-.086	.079	-.078	-.022	.833	.179	-.062	1.00

Logistic regression

A preliminary binary model was run with all 17 independent parameters in order to determine if any parameter was significant with mortality. Only Temperature in the warm trimester and Temperature in the dry trimester are significant at a significance level of 0.05 (Table 3).

Table 3 – P-value for all environmental parameter at a significance level of 0.05.

Environmental Parameters	**p-value**
Altitude	0.33
Radiation	0.18
Slope	0.28
Annual Temperature	0.13
Temperature in the cold trimester	0.44
Temperature in the warm trimester	**0.01**
Temperature in the humid trimester	0.71
Temperature in the dry trimester	**0.01**
Annual Precipitation	0.28
Precipitation in the cold trimester	0.31
Precipitation in the warm trimester	0.24
Precipitation in the humid trimester	0.24
Precipitation in the dry trimester	0.55
Available water capacity (AWC)	0.19
Potential evapotranspiration (PET)	0.09
Moisture “supply-demand” (PE)	0.23
Actual evapotranspiration (AET)	0.63

Even though only these two parameters were found to be statistically significant biologically all of them are relevant to the growth or decline of plant species. In order to include more parameters in the model the results of the Pearson correlation (Table 2) were taken into account to correctly choose. Parameters that showed high correlation coefficients (more than 90%) with more than one parameter were excluded until only parameters with no high correlation were left. Nine environmental parameters were eliminated in the following order: Annual Precipitation, Temperature in the dry trimester, Precipitation in the humid trimester, Mean annual Temperature, Altitude, Precipitation in the cold trimester, Temperature in the cold trimester, Moisture “supply-demand” (PE) and Precipitation in the dry trimester.

Only eight parameters remained as input to the binary logistic (Table 4).

Table 4 - Environmental parameters used in the Binary logistic regression model.

Environmental Parameters
Radiation
Slope
Temperature in the warm trimester
Temperature in the humid trimester
Precipitation in the warm trimester
Available water capacity (AWC)
Potential evapotranspiration (PET)
Actual evapotranspiration (AET)

With the eight remaining parameters a binary logistic model using the stepwise Backward likelihood method was developed to evaluate which combination revealed the best fit for the determination of the mortality in the maritime pine trees. In this method the first Step (Step 1) includes all independent parameters than in each Step the model progressively excludes the parameter with the highest value of p-value until the parameters left are all significant at a significance level of 0.05.

The Omnibus Tests of Model Coefficients (Table 5) is used to check if the new model, with the independent parameters included, is an improvement over the baseline model with the use of chi-square tests.

Table 5 - Omnibus Tests of Model Coefficients.

Step	Environmental parameters included	Chi-square	df	Sig (p-value)
Step 1	Radiation Slope Temperature in the warm trimester Temperature in the humid trimester Precipitation in the warm trimester AWC PET AET Constant	14.918	8	0.061
		14.918	8	0.061
		14.918	8	0.061
Step 2	Radiation Slope Temperature in the warm trimester Temperature in the humid trimester Precipitation in the warm trimester AWC PET Constant	-0.235	1	0.628
		14.683	7	0.040
		14.683	7	0.040
Step 3	Radiation Temperature in the warm trimester Temperature in the humid trimester Precipitation in the warm trimester AWC PET Constant	-1.129	1	0.288
		13.554	6	0.035
		13.554	6	0.035
Step 4	Radiation Temperature in the warm trimester Temperature in the humid trimester Precipitation in the warm trimester AWC Constant	-.751	1	0.386
		12.803	5	0.025
		12.803	5	0.025
Step 5	Radiation Temperature in the warm trimester Temperature in the humid trimester AWC	-1.254	1	0.263
		11.548	4	0.021
		11.548	4	0.021

	Constant			
Step 6	Temperature in the warm trimester	-1.798	1	0.180
	Temperature in the humid trimester AWC	9.750	3	0.021
	Constant	9.750	3	0.021
Step 7	Temperature in the warm trimester	-.941	1	0.332
	AWC	8.809	2	0.012
	Constant	8.809	2	0.012
Step 8	Temperature in the warm trimester	-1.889	1	0.169
		6.920	1	0.009
	Constant	6.920	1	0.009

The new model, Step 8, which only includes the temperature in the warm trimester, has a significantly reduced Chi-square compared to Step 1. This indicates that the new model is better at predicting the dependent variable and is statistically significant ($p < 0.05$).

The best binary logistic model for mortality estimation included only one independent parameter, temperature in the warm trimester (Table 6).

Table 6 - Variables in the equation after Backward likelihood ratio method.

Environmental Parameters	**B**	**S.E**	**Wald**	**df**	**p-value**	**Exp(B)**
Temperature in the warm trimester	0.850	0.359	5.619	1	.018	2.340
Constant	-17.274	7.392	5.461	1	.019	0.000

The values indicate that Temperature in the warm trimester is statistically significant (p-value <0.05) and therefore there is statistical evidence that it contributed to the increase of mortality. The S.E. indicates how stable the estimated coefficient is, where high values mean that the coefficient is not well estimated and low values mean a fairly precise estimate. In this case Temperature in the warm trimester was well estimated.

The equation for the best fit model to determine probability of mortality of the maritime pine trees observed in the study is:

$$Mortality\ = 0.850 \times Temperature\ in\ the\ warm\ trimester - 17.274$$

According to SPSS calculations this equation can predict 66.7% of the mortality, which is more than the 54.9% in the “null model”, the model without environmental parameters involved. That is, the model does better than chance at predicting mortality.

To measure the goodness-of-fit of the binary logistic model a Receiver Operating Characteristic (ROC) statistics needs to be calculated to validate the model (Figure 16; Table 7).

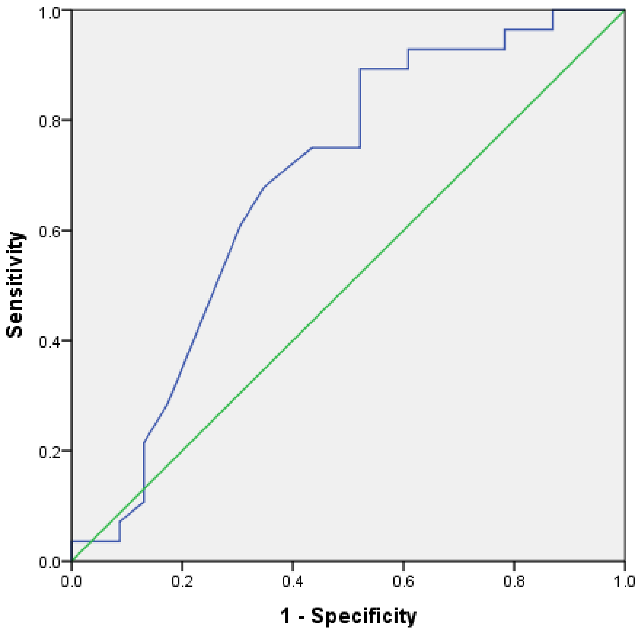

Figure 16 - ROC curve for the better fit model.

In order to evaluate if the parameters generated in the better fit model are good in predicting the maritime pine mortality, the area under the curve needs to be analyzed (Table 7).

Table 7 - Area Under the curve Results (Predicted probability).

Test Result Variable(s): Predicted probability				
Area Under the Curve	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.685	0.078	0.024	0.531	0.838

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

The area under the curve is 0.685 (>0.5) and is significant (p-value is 0.024 < 0.05), with 95% confidence interval (0.531, 0.838). This indicates that the logistic regression model determines probability of mortality significantly better than by chance.

5. Discussion

The results showed that only one parameter, Temperature in the warm trimester, was significant and can be used to predict the outcome of mortality by 66.7%. This confirmed that the methodology applied was suitable not only to predict the outcome better than by chance but also to increase the correct classification of infected sites.

The importance of spatial autocorrelation in the outcome of statistical analyses has generated different opinions. Some authors defend that spatial autocorrelations needs to be removed as it accounts for values that may be influencing the prediction model (Dormann *et al*, 2007) and inducing pseudoreplication (Hurlbert, 1984; Legendre, 1993). To deal with this problem, several methods have been developed to correct the effects of spatial autocorrelation, such as 1) Autocovariate regression; 2) Spatial eigenvector mapping (SEVM); 3) Generalized least squares (GLS); 4) Conditional autoregressive models (CAR); 5) Simultaneous autoregressive models (SAR); 6) Generalized linear mixed models (GLMM); and 7) Generalized estimation equations (GEE) (Dormann *et al*, 2007). The problem with these methods is that most of them use normally distributed data and the ones suitable for binary data showed different patterns when applied (Dormann *et al*, 2007), not reassuring that the elimination of the spatial autocorrelation improves the outcome. Other authors defend that spatial autocorrelation is ecologically significant, and if removed may omit important and relevant information in these type of studies such as ecological processes (Dormann, 2007; Austin, 2002). Dormann (2007) states that based on currently available evidence, spatial autocorrelation is relevant across all groups of organisms and all spatial scales and contains information one might not want to "correct for" (Dorman, 2007).

The results of the spatial autocorrelation analysis showed that all maps had a positive spatial autocorrelation. This might be due to the fact that environmental parameters, such as temperature, exhibit spatial autocorrelation because there is a higher probability of locations closer to each other (neighboring locations) to have similar conditions than places further apart (Cliff & Orde, 1973), and that the environmental factors can limit the dispersion of the organisms (Austin, 2002; Epperson, 2005; Karst et al., 2005; Lloyd et al., 2005; Jones et al., 2006; quoted in Dormann, 2007; Dormann et al., 2007).

Taking into account the point of view of Dorman (2007), defending the importance of spatial autocorrelation in the understanding of ecological systems, and the statistical approach of Vu *et al.* (2014), the statistical analysis in this thesis proceeded without the elimination (or partial elimination) of the spatial autocorrelation of the model.

In previous studies performed with trees in greenhouses, temperature has shown to be relevant, and that in high temperatures (25-30 °C) the decline of the infected tree is accelerated (Mendes, 2012; Colwell, 2013). In this study the outcome is in agreement with these studies and highlights that temperature is in fact an important parameter for tree mortality. In the Mediterranean climate the warmest trimester coincides with the driest one and is associated with the months of lower precipitation (Colwell, 2013). Even though the model only included temperature, this parameter might influence other parameters such as precipitation.

The increase of temperature will contribute to the acceleration of the decline of the tree especially when associated with a low water content. On the other hand, this increase provides better conditions for the development of the PWN and its vector.

From the results of this study it is not possible to know exactly if the mortality occurs from the decline of the vitality of the tree or if it is directly related to the PWN. However the results show that temperature is an important parameter in the mortality of the tree and therefore should be explored in future studies.

This study also indicates that temperature alone can be used to estimate the probability of mortality, which is an advantage to any study since it is widely available as it is measured in all weather stations. The only problem with this parameter is that the data is not recent and may not portray the present.

It has been predicted that climate change will alter the patterns of environmental parameters. It is expected that temperature will increase and in the Mediterranean climate, the climate in this study site, these warmer months correspond to the months with least precipitation (Sala et al., 2000; Allen *et al*., 2010) making pine trees more susceptible to the pine wilt disease (Colwell, 2013) since these conditions do not correspond to their optimal conditions.

6. Conclusions and future work

This study highlights the importance of high temperatures in the death of the pine trees associated with the PWN and it was possible to prove that in real conditions the parameter that influences the mortality the most is the same that was found in studies done in controlled situations.

The introduction of this parameter in the model decreases the errors associated with the determination of the mortality and demonstrates that the methods used are useful and can be used in other places to determine mortality and help assist the ongoing inspection of the spread of the disease.

Knowing that climate change will increase the temperature, the dispersion of this PWN to other areas that have not been infected, due to the absence of the optimal conditions for the settling of PWN, is a possibility.

As part of the climate change scenarios, the results are important since they allow to easily obtain values of probability of mortality.

Limitations and future work

The lack of information available about the exact places and the quantity of PWN found in each intervention site (IS) made it hard to compare the relationship of the data with the actual PWN. The fact that only the mortality basal area was evaluated and that it was not possible to collect samples from the sampling plots to classify if the death of the tree was due to the presence of the PWN, making it difficult to identify the true cause of the tree decay.

The soil data presented some limitations due to the lack of data or the different types of soil. This was an issue in the computation of several parameters in the Water Balance Toolbox, because some of them required the soil map, leading to the exclusion of these areas.

For future work there are some topics that would improve the knowledge of this issue. Tree density data for the study site and other areas of Portugal would make it possible to relate it to the places where the disease has been confirmed. The comparison of the amount of invasive species that might compete with the pine trees would also show if there is a competition for resources, making the pine trees lose their ideal condition and therefore being more susceptible to the PWN.

The determination of the effect that climate change will have on the dispersion of the PWN, due to the increase of the vulnerability of the pine forests, and how it will affect the mortality rate.

Finally, it would also be interesting to extend this study to other areas of the country, to evaluate if the environmental parameters that influence tree mortality vary from site to site or if there is a pattern, so that in the future this method can be used as a complementary way to study and detect the PWN.

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